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Cancer Development

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13. ABSTRACT (Maximum 200 Words) Prostate cancer is the most common malignancy in men. Studying the biology of prostate cancer and development of new therapies are hampered by a lack of insight into the molecular basis of the disease and appropriate animals models. The recently identified tumor suppressor gene PTEN is a promising candidate for being involved in prostate cancer since it is frequently deleted in prostate cancer, especially in advanced or metastatic forms. To study the function of PTEN in prostate cancer development, we have deleted Pten gene and generated an animal model system. Mice lacking one allele of Pten gene developed prostate abnormalities, ranging from hyperplasia to malignant carcinomas, starting from the 8 th month. To accelerate this process, we have generated Pten ^{loxp/loxp} mice, which will allow us to delete Pten specifically in the prostate glands. We are currently breeding the Pten ^{loxp/loxp} mice with prostate specific Cre transgenic mice. We have also generated a TAT-Cre fusion protein which will allow us to focally delete Pten by surgical injection into the prostate. This study will not only allow us to better understand the function of PTEN in prostate cancer, but will generate a novel animal model for possible treatment.				
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INTRODUCTION

PTEN is a tumor suppressor gene frequently deleted in many human cancers, including prostate cancers. The goal of this funded proposal is to study the function of PTEN in prostate cancer development in vivo. Since loss of PTEN function causes embryonic lethality, we proposed to specifically inactivate Pten in the prostate.

Three specific tasks should be accomplished in this study:

- I. To characterize Pten^{loxP/loxP} mouse strain;
- II. To inactivate Pten in the secretory epithelium of the prostate gland by intercrossing Pten^{loxP/loxP} and probasin Cre transgenic mice;
- III. To forcibly inactivate Pten through injection of CMV-Cre-T adenovirus into the prostate of Pten^{loxP/loxP} mice.

BODY: STUDIES AND RESULTS

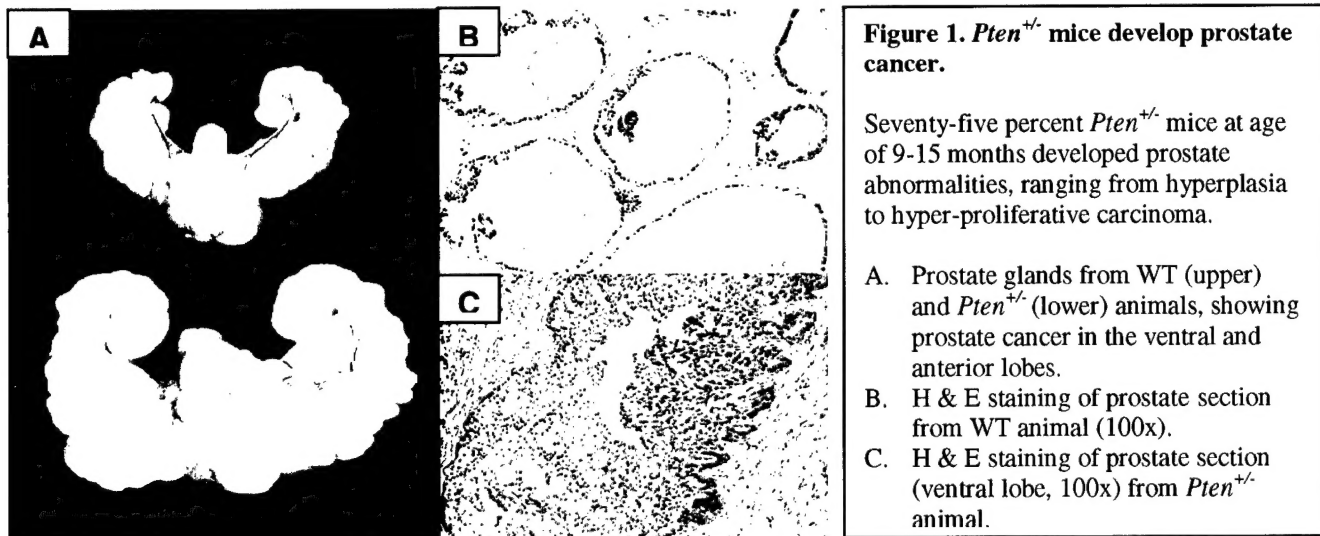
I. To characterize $Pten^{loxp/loxp}$ mouse strain

I-1. To generate $Pten^{loxp/loxp}$ mouse strain on 129svJ/C57Bl/6 and 129svJ/Balb/c backgrounds

Achieved.

I-2. To generate isogenic 129svJ $Pten^{loxp/loxp}$ mouse strain

One important consideration in generating mouse models for genetic diseases in their genetic background. Since possible influence of genetic background on prostate cancer have not been previously reported, we initially proposed to generate isogenic 129svJ mouse strain for future backcrossing to other genetic background.



During the past year, we have conducted detailed study on $Pten^{+/-}$ mice, especially the possible impacts of different genetic backgrounds on prostate cancer development. As reported by others (Di Cristofano et al., 1998; Podsypanina et al., 1999; Stambolic et al., 1998; Suzuki et al., 1998), $Pten^{+/-}$ mice under 129/C57Bl/6 develop tumors in multiple organs at early stage of adulthood (3-4 months). However, only hyperplastic changes were observed in the prostate glands of the mutant mice (Di Cristofano et al., 1998). In contrast, mice under 129/Balb/c background had rather late onset of tumor development (Lesche and Wu, unpublished results). $Pten^{+/-}$ mice under such genetic background were free of tumor for the first 6 months of their life. Interestingly, 75-85% of males developed prostate abnormalities, ranging from hyperproliferation to malignant carcinomas, starting from the 8th month (see Figure 1). The molecular mechanisms of such genetic background influence are very important and currently under investigation. On the other hand, these data suggest that establishment and maintaining the 129svJ $Pten^{loxp/loxp}$ mouse strain is no longer necessary.

I-3. To characterize $Pten^{loxp/loxp}$ mouse strain

As a prerequisite for subsequent inactivation, it is crucial to prove that introduction of loxp sites into the introns on either side of exon 5 has not effects on the function of PTEN in Pten^{loxp/loxp} mice. For this, we have followed a cohort of Pten^{loxp/loxp} mice over a period of 1.5 year. In contrast to Pten^{-/-} mice which die at early stage of embryogenesis, Pten^{loxp/loxp} mice showed no signs of abnormalities and free of tumors, suggesting that PTEN function is intact in these mice. Further study also indicated that the amount of PTEN protein produced in the Pten^{loxp/loxp} mice are comparable to the WT mice (data not shown).

II. To inactivate Pten in the prostate secretory epithelium by intercrossing Pten^{loxp/loxp} mice and probasin-Cre transgenic mice.

We have obtained probasin-Cre transgenic mice from Drs. Norm Greenberg and also signed MTA for PB-Cre4 mice (a modified probasin promoter driven Cre recombinase, see appendix) with Dr. Roy-Burman at USC. Animal breeding is in progress with a final goal to obtain Pten^{loxp/loxp}; Cre^{+/-} and Pten^{loxp/d5}; Cre^{+/-} mice.

III. To focally inactivate Pten through injection of CMV-Cre-T adenovirus into the prostate of Pten^{loxp/loxp} mice.

We have conducted a preliminary experiment by injecting CMV-Cre-T (provided by Dr. Lily Wu, UCLA School of Medicine) directly into the mouse prostates. The injection was successful and we were able to deliver approximately 1×10^9 viral particles into individual prostate gland. Excision of exon 5, which was flanked by the loxp sequences, could be detected in the injected prostate gland (data not shown). However, since the adenovirus vector used here belongs to the first generation of viral vector, significant immune response were observed in the injected mice: including enlarged surrounding lymph nodes and lymphoid cell infiltration. The immune response would complicate the outcome of this experiment. To overcome this problem, two alternative approaches were investigated:

III-1. To generate a "gut-less" adenovirus vector carrying the Cre recombinase

Dr. Lily Wu, our collaborator, is generating such vector now.

III-2. To generate a TAT-Cre fusion protein for protein transduction

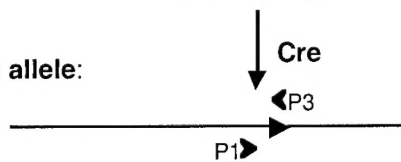
Recently, a novel technique has been discovered that might allow one to focally deliver Cre recombinase in vivo. The novel technology co-opts the interesting ability of the HIV TAT protein to cross cell membranes in a receptor-independent and endocytosis-independent manner. Although its mechanism of action is unclear, a 36 amino acid domain of TAT has been defined to mediate this phenomenon (Nagahara, 1998). By fusing this TAT domain to Cre recombinase, we hope to develop a novel molecule that will be able to delete Pten gene in vivo in a temporal and spatial controlled manner. We have generated TAT-Cre fusion protein and proved its function in tissue cultured cells. We have also injected TAT-Cre fusion protein into to ventricle of Pten^{loxp/+} mouse brain and detected exon 5 excision of the flexed-Pten locus (Figure 2). We have now produced enough TAT-Cre proteins and will inject them directly into the prostate glands of Pten^{loxp/lox} mice.

A.

Conditioned allele:



$\Delta 5$ allele:



WT:	1050bp
loxP:	1150bp
$\Delta 5$	450bp

B.

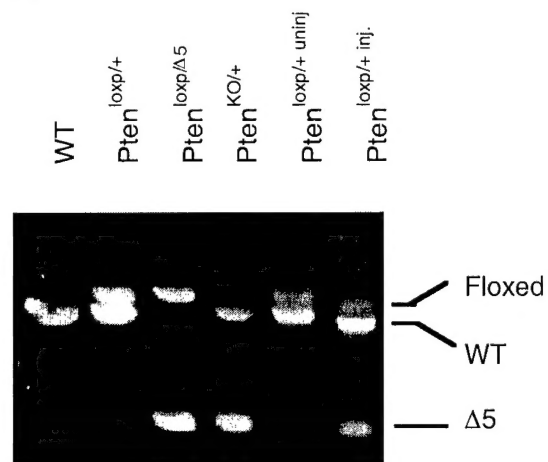


Figure 2. Conditional knock-out of floxed-*Pten* allele.

- A. *PTEN*-conditioned allele. Exon 5 of the *PTEN* gene is flanked by the loxP sequences. P1 to P3 are PCR primers. When Cre is available, exon 5 will be deleted and create a *Pten* $\Delta 5$ allele.
- B. PCR genotyping. Left four lanes are genotype controls. Lane 5 is uninjected *Pten*^{loxP/+} mouse and lane 6 is *Pten*^{loxP/+} mouse brain injected with TAT-Cre.

KEY RESEARCH ACCOMPLISHMENTS

- Established and characterized Pten conditional knock-out mouse strain: Pten^{loxp/loxp}
- Proved important role of PTEN in prostate cancer development by studying Pten^{+/-} male mice under Balb/c/129 background
- Obtained probasin-Cre transgenic mice for prostate epithelium-specific deletion of Pten
- Generated TAT-Cre fusion protein for focal deletion of Pten gene in the prostate glands

REPORTABLE OUTCOMES

- Publication: Dubey, P., **Wu, H.**, Reiter, R. and Witte, O. (2001) Alternative Pathways to Prostate Carcinoma Activated PSCA Expression, Cancer Research (in press).
- Presentation: CapCure Annual Meeting, Sept. 2000, Lake Tahoe, Nevada
- Repositories: NCI Animal Models for Human Cancers Consortium

CONCLUSION

Our study on Pten knock-out mice suggest that PTEN is a crucial tumor suppressor gene in controlling the prostate cancer development. Thus, prostate-specific deletion of Pten will provide a valuable model for studying prostate cancer, especially signaling pathways involved in prostate cancer formation. Results derived from this study will provide molecular insight to tumorigenesis in the prostate glands and possible therapeutic intervention for treatment of prostate cancers.

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APPENDICES

MTA for PB-Cre4 mice.



January 9, 2001

Office of
Technology Licensing

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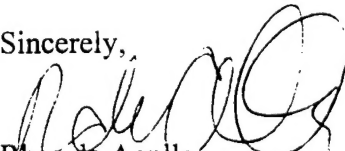
Re: Material Transfer Agreement
Probasin-Cre Mouse Line (Dr. Roy-Burman)

Dear Dr Wu:

Please find enclosed in triplicate the Material Transfer Agreement for the materials you requested from Dr. Roy-Burman. Please sign, as well as, obtain the signatures from an authorized representative and return all three (3) originals to my attention. Upon receipt of the signed originals, I will obtain the final signatures and send a fully executed original for your files.

Thank you for your interest. If you have any questions regarding this matter, please do not hesitate to contact me by telephone at (213) 743-2282 or by e-mail at deaenlle@usc.edu.

Sincerely,



Rhea de Aenlle

Enclosure

pc: Dr. Roy-Burman (w/o enclosures)

RD/sp

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academe/otl](http://www.usc.edu/academe/otl)

MATERIAL TRANSFER AGREEMENT

This Material Transfer Agreement ("MTA") is effective beginning on the date of the latter of two authorized signatures of the parties. The parties in this Agreement are the University of Southern California, the University of Manitoba (hereinafter referred to as "PROVIDERS") and University of California, Los Angeles School of Medicine (hereinafter referred to as "RECIPIENT"). The Research Material, defined below, will be provided by Dr. Pradip-Roy-Burman, Professor, USC Department of Pathology (hereinafter referred to as "PROFESSOR") to the RECIPIENT.

1. PROVIDERS agree to transfer to RECIPIENT the following Research Material: Probasin-Cre mouse line (specifically designated as PB-Cre4 mouse line) relating to USC File No. 2994.

2. This Research Material will be used by RECIPIENT solely in connection with the following evaluation research project ("Evaluation Project") described with specificity as follows:

RECIPIENT will be using the Research Material to study the function of PTEN tumor suppressor.

3. This Research Material will only be used for research purposes by RECIPIENT in their laboratories under suitable containment conditions. This Research Material will not be used for commercial purposes such as screening, production or sale, for which a commercialization license may be required. The Research Material will not be used for in vivo testing in human subjects. RECIPIENT agrees to comply with all Federal rules and regulations applicable to the Evaluation Project and the handling of the Research Material.

4. RECIPIENT agrees to treat in confidence, for a period of five (5) years from the date of its disclosure, any of PROVIDERS' written information about this Research Material that is stamped "CONFIDENTIAL." Notwithstanding this provision, RECIPIENT shall not have any obligation under this AGREEMENT with respect to information disclosed by the PROVIDERS to the extent that RECIPIENT can document that the information:

- a. was in the public domain at the time the PROVIDERS communicated such information to RECIPIENT;
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- c. was in RECIPIENT's possession free of any obligation of confidence at the time the PROVIDERS communicated such information to RECIPIENT;
- d. was rightfully communicated to RECIPIENT by a third party free of any obligation of confidence subsequent to the time the PROVIDERS communicated such information to RECIPIENT; or
- e. was developed by employees or agents of RECIPIENT without reference to any information that the PROVIDERS have disclosed in confidence to any third party.

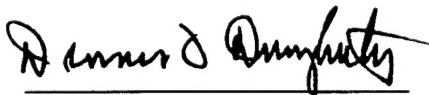
5. This Research Material represents a significant investment of the part of PROVIDERS, and is considered proprietary to the PROVIDERS. RECIPIENT therefore agrees to retain control over this Research Material, and further agrees not to transfer the Research Material to other people not under its direct supervision without advance written approval of the PROVIDERS. The PROVIDERS reserve the right to distribute the Research Material to others and to use it for its own purposes. When the Evaluation Project is completed, the Research Material will be destroyed by RECIPIENT or otherwise disposed of as mutually agreed by provider and RECIPIENT.

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PROVIDERS

UNIVERSITY OF SOUTHERN CALIFORNIA

BY:



Dennis F. Dougherty

TITLE: Senior Vice President,
AdministrationDATE: 12/13/00

DR. PRADIP ROY-BURMAN

BY: _____

DATE: _____

UNIVERSITY OF MANITOBA

BY: _____

TITLE: Vice-President (Administration)DATE: Jan 4/01

RECIPIENT

UNIVERSITY OF CALIFORNIA, LOS ANGELES
SCHOOL OF MEDICINE

BY: _____

TITLE: exec AdminDATE: 1/18/01

HONG WU, M.D., PH.D

DEPARTMENT OF MOLECULAR AND MEDICAL
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DATE: 1/18/01